Correlates of Oxidative Stress and Free-Radical Activity in Serum from Asymptomatic Shipyard Welders

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Rationale: Oxidative stress is believed to play a key role in the development of welding-induced disease.

Objectives: This study investigated the effects of welding fume exposure on correlates of oxidative stress in the serum of asymptomatic shipyard welders.

Methods: Blood samples from 197 male welders and 150 unexposed male office workers were analyzed for manganese and lead. Serum was assayed for protein, albumin, total antioxidant status (TAS), manganese superoxide dismutase (Mn-SOD), aconitase, glutathione peroxidase (GPx), heat shock protein 70, isoprostane, and reactive oxygen species, using electron spin resonance and chemiluminescence. Comparisons between welders and control subjects on biomarkers of oxidative stress were made, and evaluated for the effects of age and smoking. Associations between blood levels of manganese and lead and biomarkers were also explored.

Results: Welding was associated with increases in serum protein, GPx, aconitase, TAS, and isoprostane levels compared with control subjects. These group differences were not altered by age or smoking. In welders and control subjects, age was significantly associated with changes in albumin, TAS, chemiluminescence, GPx, and Mn-SOD. In welders and control subjects, smoking resulted in a decrease in GPx, and in a significant interaction between smoking and chemiluminescence. There were significant correlations between manganese levels in welders' blood and chemiluminescence, GPx, and Mn-SOD, and between lead levels and albumin, TAS, GPx, and Mn-SOD.

Conclusions: These results document that exposure to welding can cause changes in serum biomarkers of oxidative stress that may be valuable in clinical monitoring of disease development and in assessing whether further reduction of worker exposures is needed.

Keywords: exposure to welding fumes; lipid peroxidation; markers of oxidative stress; reactive oxygen species; serum antioxidants

Approximately 800,000 to 1,000,000 workers are employed as full-time welders worldwide (1, 2). The Bureau of Labor Statistics estimated that more than 354,300 workers were employed as welders, solderers, brazers, or in other welding-related work in the United States during 2003, and more than 2,000,000 workers were involved in some type of welding work (3). Welding generates fumes that may contain many toxic materials, including several metals (e.g., cadmium [Cd], chromium [Cr], iron [Fe], lead [Pb], manganese [Mn], and nickel [Ni]), and toxic gases (e.g., carbon monoxide, ozone, and nitrogen oxides) (2). These metals and gases have the potential to produce adverse health effects in welders.

The concentration and type of metal particulates and gases generated in welding are dependent on the composition of the filler metals, materials used in welding, welding processes, and use of flux. Fumes generated from mild steel welding usually contain more than 80% or more of iron and variable levels of Mn (0.3–1.3%), and fumes from stainless steel welding contain 20% Cr and 10% Ni (2). Shielding gases such as mixtures of helium, argon, or carbon dioxide are used to reduce oxidative reactions and protect the weld (4). Their use can produce toxic gases, such as nitrogen oxide and ozone, through photochemical reactions induced by increased ultraviolet radiation. In addition, carbon dioxide used in the shielding gas can undergo a reduction reaction and be converted to the more chemically stable carbon monoxide (2).

Welding fumes can cause a variety of adverse health effects from minor symptoms, such as headache, nausea, and metal fume fever, to severe health effects, such as occupational asthma, bronchitis, pneumoconiosis, lung cancer, and manganism (2, 5–13). Epidemiologic studies have linked adverse respiratory effects with differences in welding materials, processes, and ventilation (5–9). Particular metal components generated in welding fumes, such as hexavalent chromium (Cr[VI]) and Ni, have been linked with carcinogenesis (10, 14–18). Cr(VI) has been shown to be reduced to its lower oxidation states within the cell with the potential to generate reactive oxygen species (ROS) (18, 19). Cr and Ni compounds also exhibit mutagenic and chromosomal aberration potential (16, 17, 20). In addition, excess exposure to iron can cause several adverse health effects (21, 22). It has been shown that Cr, Ni, and Fe can produce ROS, such as hydroxyl radicals ($\cdot$OH), superoxide anion ($\cdot O_2^-$), singlet oxygen ($^1O_2$), and H_2O_2 (18, 19, 23). This increased production of ROS can trigger several key signaling events, which can provoke adverse biochemical and molecular abnormalities in the target cells, leading to disease.

The effects of welding fumes exposure have been studied both in human and in animals, but a comprehensive understanding of the biochemical and biological changes occurring in exposed populations is still unclear. Therefore, this study was focused on the identification of oxidative stress–based biomarkers associated with exposure to welding fumes. The purpose of this study was to measure levels of a number of correlates of oxidative stress markers in the serum of currently employed asymptomatic welders in comparison with unexposed white-collar workers. In addition, we also investigated whether the welding exposure duration correlated with levels of Mn and Pb in the blood of welders or with serum markers of oxidative stress. Some of the results of these studies have been previously reported in the form of an abstract (24).

METHODS

Study Population

The primary hypotheses to be evaluated in the serum of welders are based on evidence from the literature of certain well characterized

markers of oxidative stress, such as lipid peroxidation byproducts, antioxidant levels, and potential to generate ROS. In addition to these primary markers of oxidative stress, a number of exploratory serum biomarkers that are often considered targets of oxidative injury were also investigated. To explore these primary and exploratory hypotheses, we used a study population consisting of 197 healthy male welders with 1 to 33 yr of exposure history in a large ship-building industry in Ulsan, South Korea. All welders were employed full-time and used a gas metal arc-welding process with carbon dioxide as shielding gas. Welders in the study population did not work in a single specific job or work site on a regular basis but were involved in various welding-related processes, including welding, cutting, fitting, and work on the docks of the shipyard. Duration of welding-related work was generally for 8 h/d for all workers. They worked under various conditions, such as semienclosed or open areas, and were exposed to variable levels of welding fumes in all occupations. Most of the workers wore single-use disposable half-mask respirators for particulates. The control subjects consisted of 150 unexposed white-collar office workers frequency matched for age ($\pm$ 5 yr) from the same industrial complex.

Welding Fume Exposure Assessment

All ambient air samples were collected with personal air samplers (GilAir sampler; Sensidyne, Clearwater, FL) on mixed cellulose ester membrane filters (Millipore Corp., Billerica, MA) with a pore size of 0.8 μm and a diameter of 37 mm. Sampling was done for 6 h at a flow rate of 1 to 2.5 L/min. Random air samples were taken at peak working hours in the afternoon on different days during the blood-sampling period. The mean values reported here for exposure to welding fumes could therefore be considered rather typical and more representative of exposure levels of the welders studied. Samples of welding fume particulates collected on cellulose filters were analyzed by flame absorption spectrometry with a Varian 300 Plus spectrophotometer (Varian Techtron Pty, Victoria, Australia) according to National Institute for Occupational Safety and Health 7300 analytical methods for Mn, Fe, Zn, Pb, Cr, Cu, and Ni (25).

Biological exposures to Mn and Pb in welders were assessed in whole blood samples collected in the morning before work. Mn and Pb in blood samples were measured by atomic absorption spectroscopy with a Varian Spectra AA spectrophotometer (Varian Techtron Pty).

From all the welders and unexposed office workers, peripheral venous blood was collected without any anticoagulants in plain tubes and kept at room temperature for 10 min. The serum was then separated by centrifugation at 1,500 $\times$ g for 10 min and stored in plastic tubes at –80°C until separated into aliquots and assayed. The study protocol was approved by the Bioethics Committee of the University of Ulsan School of Medicine (Ulsan, South Korea) and by the Human Subjects Review Board of the National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention (Washington, DC). Informed consent was obtained from all the study participants.

Total Protein and Albumin

Total serum protein and albumin levels are valuable markers for monitoring several disease conditions and changes caused by oxidative stress (26, 27). Amino acid side chains and albumin are susceptible to oxidative damage. Total protein concentration in serum was analyzed by a Sigma Diagnostics (St. Louis, MO) method, according to manufacturer's protocol. Total serum albumin was determined by a Sigma Diagnostics method based on the reaction of albumin with bromocresol green as reported previously (28), using an autoanalyzer at an absorbance of 600 nm (Cobas Mira; Roche, Montclair, NJ). Concentrations of total protein and albumin in the serum samples were calculated from standards constructed with known amounts of protein or albumin.

Total Antioxidant Status

Total antioxidant status was measured by monitoring radical cation formation from 2,2′-azino-di-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce a radical cation with a stable blue color, which was measured at 600 nm (27, 29). The colorimetric method was programmed into a Cobas Mira autoanalyzer, using a Randox kit (Randox Laboratories, San Francisco, CA) according to a protocol reported previously (28), and data were expressed as millimoles per liter.

Aconitase

Aconitase is an important member of the citric acid cycle and is often a target of mitochondrial oxidant injury. Aconitase activity is considered a sensitive and specific indicator of oxidative injury and disease progression. Aconitase activity was measured in serum samples of welders and control subjects, using a Bioxytech Aconitase-340 assay kit, according to the manufacturer's protocol (Oxis Research, Portland, OR). The assay is based on the isomerization of citrate to isocitrate and the measurement of NADPH formed from NADP$^+$, which is proportional to aconitase activity (30). Measurements were performed with a Cobas Mira autoanalyzer (Roche) at 340 nm for 5 min at 37°C. The concentration of aconitase was expressed as milliunits per milliliter of serum.

Glutathione Peroxidase

Glutathione peroxidase (GPx) is an important antioxidant enzyme involved in the detoxification of peroxides and the protection of cells from lipid peroxidation. GPx catalyzes the reduction of H_2O_2 to water. GPx was measured in welder and control serum samples with a Cobas Mira autoanalyzer (Roche), using a detection kit programmed according to the manufacturer's protocol as reported previously (31). GPx was expressed as the amount of enzyme that transformed 1 μmol of NADPH to NADP per minute at 37°C.

Manganese Superoxide Dismutase

Activity of manganese superoxide dismutase (Mn-SOD) in serum samples was measured with a superoxide dismutase assay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's protocol. For analysis, 10-μl serum samples were treated with 190 μl of tetrazolium and 10 μl of 1 mM potassium cyanide to inhibit both Cu/Zn-SOD and extracellular SOD. The reaction was initiated by adding 20 μl of xanthine oxidase followed by incubation for 20 min at room temperature. Absorbance change was read at 450 nm, using a microplate reader. The activity of Mn-SOD (expressed as units per milliliter) was calculated from a standard curve constructed with known amounts of standards processed with samples. Mn-SOD activity was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical and was expressed as units per milliliter of serum.

Chemiluminescence

The potential of serum from welders and unexposed control subjects to cause oxidative activity by the generation of free radicals was determined by monitoring luminol-mediated chemiluminescence (CL). The reaction mixture contained 10 μl of serum and 0.1 mM luminol in 10 mM sodium phosphate buffer (pH 7.4). The reaction was initiated by the addition of H_2O_2 at a final concentration of 1 mM, using an automatic injector. CL was measured for 10 min with a microplate luminometer (Berthold Technologies, Bad Wildbad, Germany). CL under different experimental conditions was performed to serve as baseline controls for ROS generation by omitting serum, H_2O_2, or luminol, or by adding catalase, to confirm the generation of ROS. Addition of catalase resulted in the inhibition of more than 80% of CL (data not shown). The reaction was performed at 37°C, and values are expressed in relative light units.

Generation of Hydroxyl Radicals by Serum Samples

In an effort to confirm the CL studies on the generation of ROS, we conducted electron spin resonance (ESR) studies of randomly selected representative serum samples from control subjects and welders in the presence of H_2O_2 and spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO). ESR spectra, recorded 15 min after initiation of the reaction with 1 mM H_2O_2 containing 200 mM DMPO and 10 μl of serum, showed a typical 1:2:2:1 quartet signal with hyperfine splittings of $a_H = a_N = $ 14.9 G. These splitting constants of hydrogen and nitrogen indicate a DMPO·OH adduct, demonstrating the generation of ·OH radicals. The ESR signals produced by the serum samples of welders and control subjects were insignificant. However, heat inactivation of serum samples produced a significantly greater signal intensity, suggesting that serum samples from welders and control subjects contained antioxidants that inhibited ·OH radical generation. No attempt was made to obtain quantitative differences in ·OH radical generation between control and welder serum samples.

Lipid Peroxidation

Oxidation of tissue phospholipids by nonenzymatic random oxidation by free radicals produces isoprostane, and changes in isoprostane levels are considered good markers of oxidative injury (32). Using an ELISA kit (Cayman Chemical), 8-isoprostane in the serum of welders and control subjects was measured according to the manufacturer's protocol. The intensity of color produced, which was inversely proportional to the amount of isoprostane produced, was calculated from a standard curve and expressed as picograms per milliliter of serum.

Heat Shock Protein 70

An anti-human heat shock protein 70 (Hsp70; IgG/A/M) ELISA kit (Stressgen Biotechnologies, Victoria, BC, Canada) was used to determine the amount of IgG, IgA, and IgM antibodies to human Hsp70 in serum samples from welders and control subjects, according to the manufacturer's protocol. Briefly, serum samples were diluted to 1:500, using sample diluent, and 100-μl samples were added to each well and then incubated at room temperature for 2 h. Absorbance of color developed was measured at 450 nm with a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA). Concentrations of Hsp70 (ng/ml) in serum samples were calculated from a constructed standard curve with known concentrations of Hsp70.

Statistical Analysis

Data were analyzed with SAS/STAT software (version 9.1 of the SAS System for Windows; SAS Institute, Cary, NC). Outcome variables were analyzed by analysis of variance methods. Two of the variables, CL and Hsp70, were transformed by using the natural log before analysis to transform the data into a normally distributed variable to meet the assumptions of the statistical analysis. All other variables were analyzed on the basis of the original units. The primary statistical analyses compared welders and unexposed control subjects on the basis of the outcome variables without the addition of covariates. Subsequent models included age and smoking status as covariates to determine whether differences between groups were modified by these variables. This included both additive and interaction models. It was clear that age influenced many of the variables and thus separate analyses examining the effects of age were performed. Additional correlation and regression analyses were performed to examine the relationship between number of years working as a welder, serum levels of Mn and Pb, and the outcome variables in welders only. These stepwise analyses also included age and smoking as covariates. Data are presented as means with 95% confidence intervals from the primary analysis without the inclusion of covariates.

Matched Case-Control Analyses of a Subset of Study Population

To investigate the association and increases or decreases in biomarkers without the possible confounding influence of cigarette smoking and age, we explicitly matched welders and unexposed control subjects on the basis of current smoking status and age. Matching was performed with a macro program (Match) written in SAS by J. Kosanke and E. Bergstrahl and made available through the Division of Biostatistics at the Mayo Clinic (Rochester, MN). This resulted in 117 unexposed control subjects and 117 welders completely matched on smoking status, and with an average age difference of 1.07 yr. Analyses of variance as described above on oxidative stress biomarkers were then performed without adjusting for age or smoking and the results were compared with those from the entire sample.

RESULTS

Study Population

Welders (n = 197) and unexposed control subjects (n = 150) were similar with respect to age and other variables except for moderate differences in height, weight, and cigarettes smoked per day (Table 1). Clinical and hematologic studies also revealed no major underlying disease processes in either group (data not shown).

Workplace Exposure Levels

Data obtained for ambient air concentrations of these metals and welding fumes in the welding work areas are presented in Table 2. Measurements of ambient air samples taken outside the welding helmet had a mean welding fume particulate load of 13.2 ± 1.8 mg/m^3 (Table 2). These exposure levels are significantly higher than the 5-mg/m^3 Occupational Safety and Health Administration American Conference of Governmental Industrial Hygienists occupational exposure limits as an 8-h time-weighted average set in the past and currently retracted as inadequate due to the complex chemical composition of welding fumes. The Occupational Safety and Health Administration permissible exposure limit for Mn is set at a ceiling limit of 5 mg/m^3, and never to be exceeded even momentarily, to protect against eye and respiratory irritation (33). The average ratio of fume concentrations inside to those outside the helmet was 0.60 ± 0.18 (n = 8). The ratio of Mn concentrations inside to those outside the helmet was 0.61 ± 0.14 (n = 8).

Blood Mn and Pb Levels in Welders

Results of blood levels of Mn and Pb in welders in this study population are presented with reported reference values in a Korean control population (Table 3). Mn and Pb values in smokers and nonsmokers in this study population of welders were not different compared with reported studies (34–36). There were also no major differences between overall levels of Mn

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF UNEXPOSED CONTROL SUBJECTS AND WELDERS FROM KOREAN SHIPYARD

Variable	Control Subjects			Welders		
	All	Nonsmokers	Smokers	All	Nonsmokers	Smokers
n	150	75	75	197	72	125
Age, yr	35.90	36.70	35.14	34.90	40.70	31.50
	(34.7, 37.2)	(34.8, 38.7)	(33.6, 36.7)	(33.4, 36.4)	(37.6, 43.7)	(30.2, 32.9)
Height, cm	172.9*	173.40	172.40	171.20	168.80	172.50
	(172.1, 173.6)	(172.2, 174.5)	(171.3, 173.4)	(170.3, 172.0)	(167.3, 170.2)	(171.6, 173.4)
Weight, kg	71.5*	72.24	70.70	66.00	66.10	66.00
	(70.1, 72.9)	(70.4, 74.1)	(68.6, 72.9)	(64.9, 67.1)	(64.2, 68.0)	(64.6, 67.4)
Smoking, yr	—	—	12.80	—	—	12.10
			(11.4, 14.2)			(11.0, 13.20)
Cigarettes smoked per day	—	—	12.3*	—	—	14.90
			(11.0, 13.6)			(13.9, 15.9)

Data represent means and 95% confidence intervals.

* Significantly different from welder group (p < 0.05).

TABLE 2. CONCENTRATIONS OF FUMES AND METALS IN AMBIENT AIR SAMPLES FROM VARIOUS WELDING SITES

Fume	Mn	Fe	Zn	Pb	Cr	Cu	Ni
13.2 (1.8)	1.1 (2.0)	3.3 (2.0)	2.0 (1.8)	0.005 (2.1)	0.006 (1.9)	0.009 (1.9)	0.004 (3.2)

Concentrations are expressed as milligrams per cubic meter. Data represent geometric means and geometric standard deviations.

and Pb between welders in this study population and reported reference values in a general Korean control population (34–36).

Group Differences in Oxidative Stress Biomarkers

Means and 95% confidence intervals for welders and unexposed control subjects for oxidative stress biomarkers are shown in Table 4. Differences between welders and unexposed control subjects were apparent for several of these biomarkers including protein, GPx, aconitase, total antioxidant status (TAS), and isoprostane levels. These differences remain unchanged after adjustment for age and smoking status, indicating that these differences are due to occupational exposure to welding fumes and not the result of the potentially confounding variables of age or smoking. This is further supported by the results of the subsequent subset analysis of matched 117 welders and 117 unexposed control subjects. Although the actual values of the estimates are altered, the results of these analyses were in complete concordance with the results from the entire sample. All group differences observed in the entire sample were also observed in the subset.

Effects of Smoking on Oxidative Stress Biomarkers

The potential modification role or confounding effects of smoking on oxidative stress biomarkers were assessed by univariate and multivariate regression models that included interaction between age and smoking status. There was a significant effect of smoking on GPx levels ($p = 0.039$), with consistent lower levels of GPx in current smokers (379 ± 7.9 mU/ml) compared with nonsmokers (403.8 ± 9.0 mU/ml). The effect of smoking in the model did not alter the difference between welders and control subjects. On the other hand, there was a significant interaction between smoking status and occupational exposure to welding for CL ($p = 0.045$). Subsequent analysis indicated that nonsmoking unexposed control subjects had the lowest level of all groups, and were significantly different from nonsmoking welders. There was no difference between smoking control subjects and smoking welders.

Effects of Age on Oxidative Stress Biomarkers

Similar to smoking, the modifying or confounding effects of age on measures of oxidative stress were assessed by multivariate statistical analyses. There were no significant interactions between age and group, indicating that any changes in these vari-

ables due to age are occurring in both groups. There were significant main effects of age on albumin, TAS, CL, GPx, and Mn-SOD. Examination of the slopes of the age regression lines for oxidative stress biomarkers against age (Table 5) indicated that albumin, TAS, and Mn-SOD showed decreases with increasing age, whereas GPx and CL showed increases with respect to age. The inclusion of age into the models did not alter the differences between welders and control subjects.

Effects of Exposure Duration on Oxidative Stress Biomarkers

Possible exposure–response relationships between measures of oxidative stress and the number of years an individual worked as a welder were examined by univariate and multivariate analyses. These analyses included levels of Mn and Pb as surrogate markers of exposure, and were available only for welders. Welding years was significantly associated with albumin, GPx, and Mn levels in univariate models (data not shown). However, given the necessary correlation between age and the number of years an individual worked as a welder, additional analyses were performed including age as a variable. In each case, the inclusion of age resulted in the loss of significance for welding years. Subsequent sequential F tests indicated that there is no evidence that welding years can account for any additional variance in the outcome variables beyond that accounted for age alone. In addition, a scatter plot analysis of welders in the 1- to 10-yr exposure group showed no trends in kinetics on any of the markers with increasing duration of exposure in the first 10 yr.

Associations between Mn and Pb with Markers of Oxidative Stress

Regression analyses were performed with Mn or Pb levels as a predictor variable for measures of oxidative stress (Table 6). There were significant positive associations between Mn levels with CL, and GPx, as well as negative associations between Mn levels and Mn-SOD. There were significant positive associations between Pb levels with albumin, TAS, and Mn-SOD, and a negative association between GPx and Pb.

Generation of Hydroxyl Radicals

As a more specific measure of ·OH generation by the serum samples from welders and unexposed control subjects, ESR measurements were made in the presence of spin trap DMPO.

TABLE 3. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS FOR MANGANESE AND LEAD IN STUDY POPULATION OF WELDERS AND REPORTED REFERENCE LEVELS IN UNEXPOSED CONTROL POPULATIONS

	All Welders	Nonsmokers	Smokers	Reference Levels* (*Reference no.*)
Mn, μg/L blood	1.44 (1.39, 1.49)	1.52 (1.44, 1.62)	1.39 (1.33, 1.45)	1.28 ± 0.27 (34)
Pb, μg/L blood	5.27 (5.1, 5.4)	5.08 (4.47, 5.45)	5.38 (5.13, 5.64)	1.14 ± 2.43 (35)
				5.73, geometric mean (36)

* Reported reference levels are from a general population of nonwelders.

TABLE 4. MEANS AND 95% CONFIDENCE INTERVALS FOR SERUM MEASURES OF OXIDATIVE STRESS IN WELDERS AND CONTROL SUBJECTS

Variable	Control	Welder
Aconitase*	30.2 (25.0, 35.4)	51.3 (47.3, 55.3)
Albumin	56.9 (56.3, 57.5)	56.3 (55.8, 56.8)
Total antioxidant*	0.89 (0.87, 0.90)	0.95 (0.94, 0.97)
Log chemiluminescence	12.8 (12.7, 12.9)	12.8 (12.7, 12.9)
Glutathione peroxidase*	367.6 (350.1, 385.2)	411.4 (396.1, 426.7)
Log Hsp70	11.9 (11.84, 12.0)	11.9 (11.86, 12.0)
Isoprostane*	28.4 (25.5, 31.2)	69.1 (66.6, 71.6)
Mn-SOD	0.65 (0.60, 0.70)	0.68 (0.63, 0.72)
Protein*	80.0 (78.7, 81.3)	85.8 (84.6, 86.9)

Definition of abbreviations: Hsp70 = heat shock protein 70; Mn-SOD = manganese superoxide dismutase.

* Significantly different between groups.

Heat-inactivated serum samples showed a characteristic ·OH radical signal that was identified as the DMPO/OH adduct with characteristic splittings (data not shown). No attempt was made to identify quantitative differences between the serum signal intensities of welders and unexposed control subjects.

Welding Exposure–Effect Relationships with Markers of Oxidative Stress in Frequency-matched Population

Because age and smoking had a significant effect on some of the outcome variables, we compared 117 welders and 117 unexposed control subjects frequency matched for age and current smoking status. These analyses found significant welding exposure–associated differences between unexposed control subjects and welders in the same five of the nine oxidative stress–induced biomarkers (Figure 1). From these results it is apparent that age and cigarette smoking are probably not contributing to the 2.4-fold increase in isoprostane and 1.5-fold increase in aconitase and the moderate increases in TAS, GPx, and protein. From these comparisons and the statistically significant differences, we believe that the changes in oxidative stress markers observed are caused by exposure to welding fumes and not by differences due to sampling.

DISCUSSION

This investigation was undertaken to explore oxidative stress–based biomarkers associated with disease development on expo-

TABLE 5. LINEAR REGRESSION COEFFICIENTS AND 95% CONFIDENCE INTERVALS, AND CORRELATION COEFFICIENTS, ON AGE FOR WELDERS AND CONTROL SUBJECTS

Variable	Age (10-yr increments)	Correlation
Aconitase	0.41 (−3.1, 3.9)	0.014
Albumin	−0.63* (−1.0, −0.22)	−0.160*
TAS	−0.01* (−0.03, −0.003)	−0.129*
Log CL	0.07* (0.004, 0.13)	0.118*
GPx	19.1* (7.0, 31.3)	0.165*
Log Hsp70	−0.04 (−0.09, 0.02)	−0.067
Isoprostane	−2.6 (−5.5, 0.40)	−0.091
Mn-SOD	−0.05* (−0.08, −0.01)	−0.133*
Protein	−0.94 (−1.87, −0.0003)	−0.105*

Definition of abbreviations: CL = chemiluminescence; GPx = glutathione peroxidase; Hsp70 = heat shock protein 70; Mn-SOD = manganese superoxide dismutase; TAS = total antioxidant status.

* Slope or correlation coefficient significantly different from 0.

sure to welding fumes so that in prospective investigations it would be possible to ascertain occupational exposure–associated changes in oxidative biomarkers. A second aim was to investigate whether any of the correlates of ROS-induced changes measured in serum show a parallel corresponding correlation with blood Mn or Pb as a surrogate for exposure to welding fumes.

Exposure to welding fumes in occupational settings causes a variety of biological effects in welders. Chronic respiratory effects associated with exposure to welding fumes are well documented and include bronchitis, occupational asthma, lung function changes, pneumoconiosis, and an increase in lung cancer incidence (2, 37–40). Neurologic, reproductive, and dermatologic effects of exposure to welding fumes are also widely studied. The welding fumes generated during welding processes contain many toxic metals, such as Cr, Fe, Mn, and Ni. The vaporized metals become oxidized in air and are then inhaled. Different metal components of welding fumes have diverse intrinsic toxic biological properties. Several metals commonly present in welding fumes, including Cr, Fe, Mn, and Ni, are capable of generating ROS via Fenton or Fenton-like reactions, resulting in oxidative stress (2, 41–44). Consequently, the increased generation of ROS has been shown to disrupt biochemical homeostasis, resulting in lipid peroxidation, DNA damage, depletion of sulfhydryls, and altered calcium homeostasis (23). *In vitro* studies support the hypothesis that soluble fractions of the welding fumes cause enhanced production of ROS with concomitant depletion of antioxidants mediating proinflammatory responses in alveolar epithelial cells by increased expression of interleukin-8 (45). Therefore, quantitative measurements of markers of oxidative stress in the serum of welders may be valuable in monitoring changes caused by exposure to welding fumes.

A potential interaction between smoking and increased ROS generation and a decrease in GPx were evident in unexposed control subjects and welders. Significant group differences between the welders and unexposed control subjects on several markers of oxidative stress were apparent in protein, GPx, aconitase, TAS, and isoprostane levels. These results are consistent with previous findings in human and animal experimental studies showing a significant increase in lipid peroxidation caused by welding fume exposure (46, 47). Other group differences in exploratory markers such as protein, GPx, and aconitase associated with welding were more pronounced in welders with increasing exposure. Serum protein and albumin are markers of pulmonary injury and potential targets of oxidative injury. An increase in the level of albumin is often associated with endothelial injury to the alveolar–capillary barrier. In welders there was a generalized trend of declining albumin levels with increasing welding years that was also influenced by age. However, albumin is considered a "sacrificial" antioxidant of the extracellular body fluids and the consequences of its damage do not affect cellular functions (48). Its turnover rate is high and the loss by oxidative stress is often not reflected in the serum. It is therefore likely that the decline noted in welders may have resulted from the combined effects of welding-induced oxidative stress and age. On the other hand, comparison of serum protein levels in all welders relative to unexposed control subjects showed a significant (p = 0.001) increase in welders. This may be considered a general marker of declining health in welders suggestive of high levels of immunoglobulins. An increase in the level of proteins is also often associated with many other disease conditions. Serum proteins are also considered targets of oxidative injury and they are often denatured by ROS (49). Depletion of these primary antioxidant defenses and the inability of welders to upregulate in parallel with increased oxidant generation may facilitate disease development. The magnitude of these changes with continued exposure to welding provides further indication of the total

TABLE 6. LINEAR REGRESSION COEFFICIENTS AND 95% CONFIDENCE INTERVALS, AND CORRELATION COEFFICIENTS, FOR MANGANESE AND LEAD IN SERUM FROM WELDERS

Variable	Regression Coefficient (95% CI)		Correlation Coefficient	
	Mn	Pb	Mn	Pb
Aconitase	3.5 (−9.1, 16.0)	−0.62 (−3.5, 2.3)	0.042	−0.032
Albumin	−1.41 (−3.0, 0.15)	0.54* (0.19, 0.89)	−0.127	0.211*
TAS	−0.003 (−0.04, 0.03)	0.01* (0.006, 0.02)	−0.01	0.236*
Log CL	0.46* (0.25, 0.68)	0.013 (−0.04, 0.06)	0.299*	0.036
GPx	51.4* (11.1, 91.7)	−24.0* (−32.8, −15.3)	0.177*	−0.362*
Log Hsp70	0.11 (−0.09, 0.32)	0.003 (−0.04, 0.05)	0.077	0.009
Isoprostane	7.0 (−1.4, 15.4)	−0.95 (−2.9, 0.97)	0.117	−0.069
Mn-SOD	−0.15* (−0.28, −0.02)	0.03* (0.003, 0.06)	−0.164*	0.153*
Protein	−1.38 (−5.2, 2.45)	1.97* (1.14, 2.80)	−0.052	0.318*

Definition of abbreviations: CI = confidence interval; CL = chemiluminescence; GPx = glutathione peroxidase; Hsp70 = heat shock protein 70; Mn-SOD = manganese superoxide dismutase; TAS = total antioxidant status.

* Indicates slope or correlation coefficient significantly different from 0.

disruption of homeostasis. Antioxidant enzymes are much lower in serum compared with intracellular levels and blood proteins and albumin are therefore subjected to greater oxidative stress than are intracellular fluids (48).

A significant increase in aconitase, a mitochondrial enzyme vulnerable to oxidant injury, was contrary to our expectations. Paradoxically, this increase in aconitase may have resulted from damage to mitochondrial aconitase with increased oxidative

stress caused by welding exposure with a parallel increase in serum. The antioxidant enzyme GPx increased significantly in welders compared with unexposed control subjects. This is contrary to a report on the level of antioxidant enzymes, plasma vitamins C and E in cement plant workers, demonstrating a 51% decrease in GPx and a 44% decrease in SOD compared with control subjects (50). Similar results for serum SOD, GPx, and catalase were also reported in oxidative stress induced by
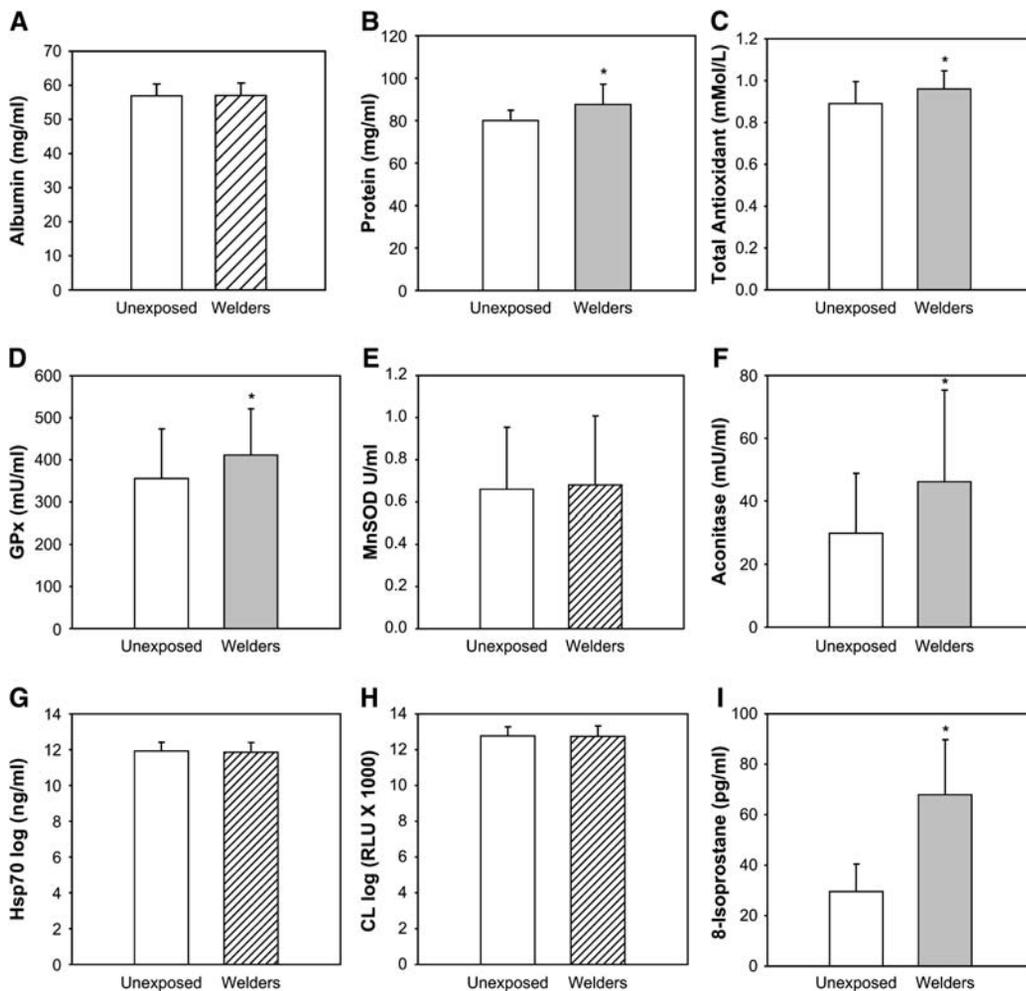
Figure 1. Effect of welding fumes exposure on serum biomarkers in a subgroup of 117 welders and 117 unexposed control subjects (*open columns*) explicitly matched for age and current smoking status. (*A*) albumin, (*B*) protein, (*C*) total antioxidant status, (*D*) glutathione peroxidase, (*E*) manganese super oxide dismutase, (*F*) aconitase, (*G*) heat shock protein (log), (*H*) chemiluminescence (*I*) 8-isoprostane. Aconitase, total antioxidant status, glutathione peroxidase, isoprostane, manganese superoxide dismutase, and protein were highly significant (p = 0.0001) in welders (*shaded* or *hatched columns*) compared with unexposed control subjects.

occupational sulfur dioxide exposure resulting in increased lipid peroxidation byproducts (51). GPx is involved in the removal of toxic H_2O_2 by converting it to water, thereby limiting lipid peroxidation. It is possible that prolonged oxidant stress would upregulate these antioxidant enzymes and this indeed may be the case with GPx in welders.

It is believed that tightly controlled levels of antioxidant enzymes may protect cells against oxidants in normal or adverse health conditions. Total antioxidant status and GPx were significantly increased in the welders. This suggests that the increased oxidative stress by welding fumes triggers the upregulation of defenses to protect cells. Contrary to these findings, Li and coworkers (46) reported in a study of 37 automobile welders a 24% decline in erythrocyte SOD activity. It is likely that such selective biochemical responses may have resulted to cope with the extent and severity of oxidant burden and can be expected to be characterized by differential regulation of individual antioxidants.

Inducible Hsp70 is present at basal levels in human serum and is often upregulated in response to stress, toxic agents, ROS, heat, and cold (52). Therefore, Hsp70 was evaluated as a potential biomarker of exposure to welding fumes. In welders and office workers, Hsp levels in serum were not influenced either by exposure to welding fumes or cigarette smoking.

In the present study, there were some positive correlations between Mn levels in the blood of welders and the potential to generate ROS, albumin, GPx, and Mn-SOD. These correlations were not consistent with increasing welding exposure because Mn is not a valuable surrogate for welding fume exposure. Similar positive correlations with blood Pb levels and albumin, protein, TAS, and Mn-SOD, and a negative correlation with GPx, were observed. Because blood levels of Mn and Pb are likely to be influenced by diet and environmental exposures, and because of their short biological half-life, a direct relationship between these surrogates of welding exposure and markers of oxidative injury could not be established.

Mn, a component of most welding fumes, has been reported to be an environmental toxic metal implicated in central nervous system injury and manganism (8, 11–13, 53). Mn can produce free radicals at cytotoxic levels causing oxidative stress and neurodegeneration (54). Mn is transported in the blood stream and is able to cross the blood–brain barrier through specific carriers (55). In this study population of welders, the mean Mn concentration in the blood was 1.5 ± 0.4 µg/dl and the mean ambient exposure concentration in the air was 1.1 ± 2.0 mg/m³. However, Mn has been shown to inhibit iron-induced lipid peroxidation (56) and is a constituent of an important antioxidant enzyme, Mn-SOD.

The results presented in this study provide support for the use of ROS-mediated changes in antioxidant status, free-radical generation potential, and resulting lipid peroxidation by products and antioxidants in serum as potential biomarkers of exposure to welding fumes. Evidence supports the possible usefulness of such biomarkers as indicators of disease in welders when the confounding effects of age and cigarette smoking are controlled. This was further explored in a subset analysis of 117 welders explicitly matched on smoking status and age with 117 unexposed control subjects (Figure 1). It is likely that the apparent 2.4-fold increase in isoprostane, the 1.5-fold increase in aconitase, and the moderate increases in TAS, GPx, and protein may have resulted from the occupational exposure to welding fumes. Because several other confounders including body mass index, diabetes, hypercholesterolemia, hypertension, and obesity are reported to be associated with serum levels of isoprostane, we investigated the influence of body mass index on isoprostane levels in the study population of unexposed control subjects and

welders and found no differences. On the basis of these results it is clear that occupational exposure to welding fumes is likely the major contributing influence in the changes of these oxidative stress–induced biomarkers.

In conclusion, our studies demonstrate that exposure to welding fumes enhanced the generation of ROS and induced oxidative stress, causing some oxidant damage in target cells and resulting in changes in certain well characterized and exploratory markers of oxidative stress. The disruption of homeostasis induced by oxidative stress may promote the development of a disease state with continued occupational exposure to welding fumes. Prospective studies using these noninvasive biomarkers of oxidative stress combined with close clinical monitoring of disease are warranted to understand the mechanisms of welding-related disease development and to assess whether there is a need for further reduction of worker exposure to welding.

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